Translocating peptides and proteins and their use for gene delivery

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A dramatic surge in the development of peptides for gene delivery *in vitro* and *in vivo* has been witnessed in the past decade. A better understanding of the structural and mechanistic properties of peptides has been an important step for the rational design of optimal peptide-based gene delivery systems. Research has focused on the design of short synthetic peptides that overcome both extracellular and intracellular limitations of other gene delivery systems by binding reversibly and condensing DNA, specifically targeting cells and/or tissues, rapidly releasing plasmids into the cytoplasm and mediating efficient nuclear translocation.

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Abbreviations
INF HA_2 subunit of influenza hemaglutinin
NLS nuclear localisation signal

Introduction

In the past decade, the development of gene therapy technology has focused in part on the design of new methods for gene delivery into cells. The major challenge resides in the design of vectors that can overcome the low permeability of the cell membrane for nucleic acids and improve intracellular trafficking and nuclear delivery of genes into target cells with minimal toxicity. Although viral vectors remain the most efficient gene transfer systems, safety concerns regarding their use in humans has made non-viral delivery systems more attractive [1,2,3••,4]. Furthermore, non-viral systems present several advantages over viral systems in that they are simple to use, easy to produce, do no induce specific immune responses and are less cytotoxic [3••,4].

Non-viral delivery systems can be divided into two main groups: cationic lipids [5–7] and polymeric DNA-binding cations [7,8••]. Over the past five years, substantial progress has been made in the development of peptide-based gene delivery systems that can overcome both extracellular and intracellular limitations of other non-viral systems, such as cell targeting, endosome lysis and nuclear translocation. The goal was to design short synthetic peptides that would mimic and act as efficiently as viruses for gene delivery without their limitations. This short review will highlight the characteristics of peptides that have been designed to address these requirements, and will discuss why the understanding of both the structural and mechanistic properties of these peptides is essential for the improvement of *in vivo* gene therapy.

Rules for the design of peptide-based gene delivery systems

The design of an 'ideal' peptide-based delivery system should satisfy four major requirements (Figure 1): firstly, bind reversibly and condense DNA; secondly, present cellular and/or tissue specificity essential for *in vivo* gene delivery; thirdly, exhibit membrane fusogenic or disruptive activities; and finally, promote nuclear translocation of plasmids.

DNA condensing motifs

As the size and the charge of peptide–DNA complexes can be the source of cytotoxicity, antigenicity and insolubility, a proper balance should be found between their size and stability [3••,4,8••]. On the one hand, peptides must form highly stable complexes with DNA in the presence of cell culture media, and on the other hand DNA condensation must be reversible to enable release of the plasmid for gene expression.

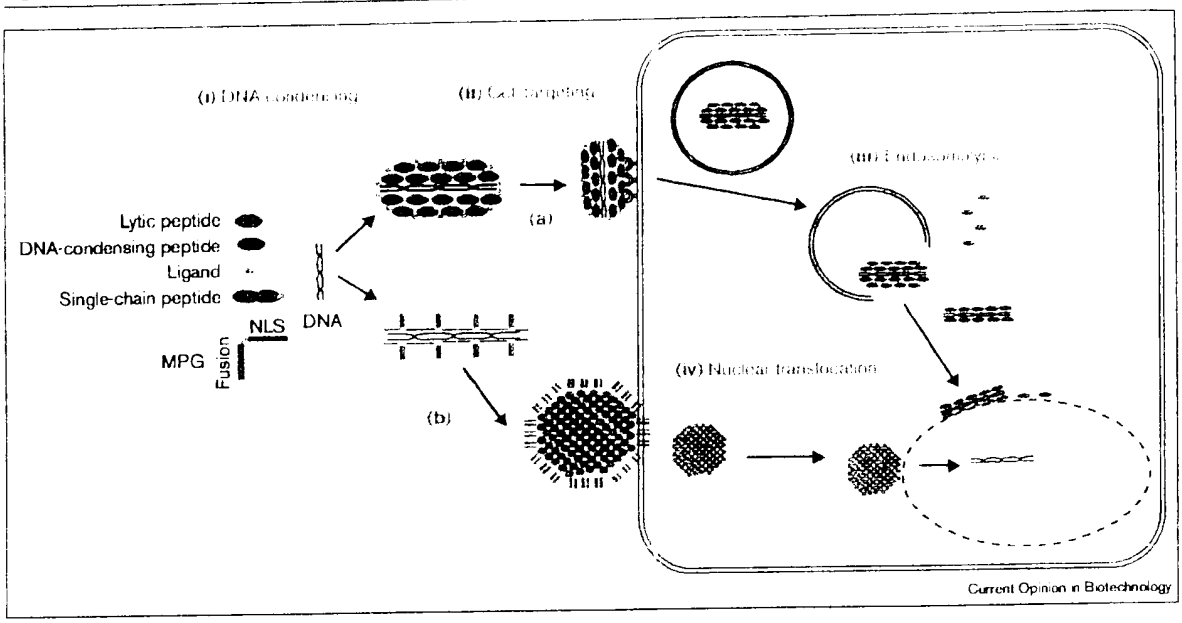
Various polyamines of different lengths and structures, such as poly-L-lysine, poly-L-arginine, spermidine, spermine, dendrimers, protamine, polyethyleneimine and poly(α,γ, diaminobutyric acid), have been proposed for *in vitro* gene transfection [8••,9–12]. Several studies using polycationic reagents, such as poly-L-lysine, have demonstrated that the maximal number of polyionic sites for cooperative binding is approximatively eight [13,14,15••]. Longer sequences were found to be less efficient, mostly because of irreversible DNA precipitation and cytotoxicity [14,15••].

In order to stabilize peptide–DNA complexes and to reduce their size, a series of modifications of DNA-condensing peptides have been proposed. Addition of hydrophobic groups or of tryptophan residues improves DNA binding. The nature of uncharged and basic amino acids, the length of fatty acid chains covalently attached to the peptide and carboxy-terminal modifications can have a significant influence on DNA binding and gene transfer efficiency [8••,16–18]. In addition, oxidation of cysteine residues in DNA-condensing peptides has been shown to generate inter-peptide disulfide bonds, which produce small particles with DNA and prevent dissociation of peptide from DNA [17]. Last, but not least, the structural organisation of the peptide is a key factor in compacting DNA, for example, for amphipathic α-helical peptides [8••,16].

Active targeting

In order to interact specifically with the surface of cells and thus deliver genes to specific cell types *in vivo*, gene delivery requires active targeting. The key is finding appropriate ligands for receptor-mediated endocytosis that

Figure 1



The four major steps in peptide-based gene delivery. **(I)** DNA binding and condensing, **(ii)** cell targeting, **(iii)** endosomal release of the plasmid and **(iv)** nuclear targeting. The two strategies for peptide-based delivery systems. **(a)** Dependent on endocytosis: self-assembly of several peptides or single chain peptide. **(b)** The non-endosomal pathway: single chain peptide (MPG).

can be incorporated into the formulation with a minimal loss of affinity and specificity. Several small peptides or epitopes have been described, most notably the tri-peptide motif RGD, found in extracellular matrix proteins, which targets integrin receptors specifically [8••,20,21]. RGD-containing peptides associated with poly-L-lysine or lipid formulations significantly improve the delivery of plasmids into specific cell lines both *in vitro* and *in vivo* [20–22]. Another targeting approach is to use small ligands, such as glycoside which targets mainly hepatocytes, covalently linked to one of the components of the plasmid complex [23,24]. Both *in vitro* and *in vivo*, oligosaccharides complexed to short poly-L-lysine peptides were shown to enhance endocytosis of plasmids specifically to the liver, thanks to the presence of receptors on hepatocytes that recognize galactosilated or mannosylated peptides [24,25].

Endosomolysis

Once carrier–plasmid complexes have been taken up by cell adsorption or by receptor mediated-endosomes, the next limiting step is the release of plasmids from endosomes before their degradation [26]. Viral genomes are released by activation of viral proteins upon acidification of the endosome. Active membrane sequences are located at the amino-terminus of such viral proteins and contain alternating clusters of hydrophobic and hydrophilic residues forming an amphipathic α-helical structure. The mechanism of membrane destabilisation by endosomolytic peptides may involve either membrane rupture or pore formation [26,27].

A number of α-helical peptides with pH-dependent fusogenic and endosomolytic activities have been described to facilitate lysosomal degradation before the contents of the endosomes are delivered to lysosomes and consequently increase transfection efficiency [8••,26,27]. Such peptides, which mimic entry of viruses into cells, are random coils with no lytic activity at pH 7.0 but become endosomolytic at pH 5.0 by adopting an amphipathic α-helical conformation that interacts with the phospholipid membrane to induce fusion and/or lysis [28,29]. The most commonly used synthetic fusogenic peptide is derived from the amino-terminal sequence of the HA_2 subunit of influenza hemaglutinin (INF; GLFEAIAGFIENGWEGMIDGGGC [amino acid single letter code]) [29,30]. Another commonly used amphipathic peptide is GALA (WEAALAEALAEALAEHLAEALAEALEALAA) [31]. INF peptides and GALA have been shown to increase the transfection efficiency when associated with poly-L-lysine–DNA, condensing peptide–DNA, cationic lipids, poly-ethyleneimine or polyamidoamine cascade polymers [30–33]. Gottschalk *et al.* [13] have designed the pore-forming synthetic peptide JTS1 (GLFEALLELLESLWLLEA). The hydrophobic face of JTS1 induces self-association and results in the formation of pores on one side of the endosomal membrane, leading to its rupture. Monsigny and co-workers [34] have proposed the peptide H_5WYG, whose membrane activity is based on the protonation of the imidazole ring of histidine. In contrast to the peptides described above, this peptide adopts an α-helical structure at pH 7.0 and permeabilizes cells as a random coil at pH 6.5 [34].

Finally, it should be noted that some peptide-mediated transfection systems do not proceed through the endosomal pathway [35,36••]. These peptides penetrate into the cytoplasm immediately at neutral pH.

Nuclear localization sequence

Most of the peptide formulations containing condensing and lytic peptides are able to transfect cultured dividing cells with a reasonable efficiency, but not quiescent cells. The dependency on the mitotic activity of the cells is primarily because of the inability of most non-viral gene delivery systems to translocate plasmids into the nucleus of non-dividing cells and remains a major problem. This parameter should therefore be taken into account in the design of new peptide delivery systems. The structure and molecular mechanisms of nuclear transport have been described in a series of reviews [37,38]. The nuclear transport process is facilitated by a nuclear localisation signal (NLS), which are short sequences that have been identified as generally one (monopartite) or two (bipartite) clusters of four or more basic amino acids (lysine or arginine) [37–39].

The NLS from the large tumor antigen of Simian virus 40 (PKKKRKV) is one the most used in non-viral gene delivery systems [35,39,40]. Peptides or proteins containing NLS, whether complexed to lipids or not, are used to improve nuclear transport through electrostatic binding of plasmids [8••]. The non-covalent attachment of NLS to DNA is preferable as this enables complete release of DNA from the NLS peptide after nucleocytoplasmic transport. In the case of chimeric proteins containing a DNA-binding domain and an NLS, competition between these two moieties for DNA binding is prevented, and the accessibility of the NLS enhanced, by using neutral or anionic NLSs, or a longer NLS sequence with negatively charged residues upstream or downstream. A second approach is the covalent attachment of signal peptides to plasmid DNA to improve its nuclear localization [41–43]. Finally, it should be noted that only one NLS attached to the end of a plasmid is the most efficient structural presentation, as multiple NLSs inhibit nuclear translocation [43].

Peptide-based delivery systems

The challenge in peptide-based gene delivery systems is to define the ideal formulation that satisfies most of the major requirements described above. Two strategies have been used so far: self-assembly of several peptides or design of a single peptide containing DNA-condensing, membrane destabilizing and NLS domains (Figure 1). In practice, the major problem resides either in forming a multicomponent DNA carrier in an orderly fashion or in the choice of sequence organisation of the different components in a single chimeric peptide.

Various plasmid formulations containing NLS, lytic and condensing peptides have been shown to be efficient for gene delivery *in vitro* [4,8••]. Endosomolytic peptides have been successfully incorporated into condensed plasmid complexes by non-covalent association with condensing peptides. This self-association is controlled by electrostatic interactions between a negatively charged fusogenic peptide and a positively charged condensing peptide. For example, the use of JTS1 peptide non-covalently associated with the cationic DNA-condensing peptide $YKAK_8WK$ (K8) dramatically improved transfections *in vitro* [13]. Competition can occur, however, between DNA and lytic peptides for the condensing peptides, which decreases the stability of the formulation in cell culture media. In the case of the JTS1/K8 formulation, improvement was achieved by attaching two palmitoyl groups to each peptide, thus creating additional contacts between the two peptides, via their palmitoyl groups. Addition of a palmitoyl group at the amino-terminus of a 12-mer DNA-condensing peptide (SPKRSPKRSPKR) associated to an INF-like fusogenic peptide also improves tranfection efficiency [44].

Another interesting approach is the design of a single peptide molecule carrying the different activities required for gene delivery. Elaboration of such chimeric peptides is not simple, however, as the structure, flexibility and accessiblity of the different domains are important parameters to be considered. Peptides containing two active domains have been used successfully for *in vitro* gene delivery, mainly with a DNA-condensing and membrane destabilizing sequence. One single-chain cyclic peptide derived from Gramicidin S was shown to condense DNA and destabilize membranes efficiently, but was poorly efficient in gene delivery mainly because of the poor flexibility of the two domains [45]. Wyman *et al.* [46] described a cationic amphipathic peptide KALA (WEAKLAKALAKALAKHLAKALAKALKACEA), which condenses DNA, destabilizes membranes and facilitates gene delivery in different cell lines *in vitro*. Similarly, Niidome *et al.* [47] described two series of cationic amphipathic helical oligopeptides derived from the 24 amino acid peptide $(LARL)_6$ and the Hel-peptide (KLLKLLLKL-WLKLLKLLL), which also present a hydrophobic/hydrophilic balance in the amphiphilic structure. These peptides exhibit a large hydrophobic region that is essential for transfection efficiency as it stabilizes the association with DNA and disrupts the endosomal membrane [47,48••]. The amphipathic helical oligopeptides seems to be promising for the design of potent *in vivo* gene delivery systems; however, problems of cytoxicity and serum sensitivity still remain to be overcome.

A series of single-chain peptides based on poly-L-lysine sequences and containing fusogenic sequences have been developed. Midoux and Monsigny [49] have exploited the protonation of histidine residues for gene delivery by producing cationic poly-L-lysine polymers partially substituted with histidyl residues [49]. Avrameas *et al.* [50••] have also proposed an interesting peptide concept by coupling an anti-DNA antibody (VAYISRGGVSTYYS-DTVKGRFTRQKYNKRA) with a 19 lysine residue

sequence, which delivers plasmids into cells in the presence of serum. The use of cell-penetrating anti-DNA antibodies should be noted as an interesting approach to control cell specificity for gene delivery, as the antibodies are also screened for their cell specificity. Schuster *et al.* [51••] have described a single-chain peptide containing an asialoorosomucoid-poly-lysine coupled with a fusogenic peptide derived from the G protein of vesicular stomatitis virus (KFTIVFPHNQGHWKNVPSNYHYCP), which promotes gene delivery in liver both *in vitro* and *in vivo*.

Cell-specific targeting is one of the main points to be addressed for *in vivo* gene delivery. For this purpose, a series of specific ligands have been incorporated covalently or non-covalently into a poly-L-lysine-containing formulation [20–24]. However, a proper balance between DNA binding and targeting of the template in the formulation should be achieved when using multi-chimeric proteins or peptides. Recently, the cationic polymer galactose-poly(L-ornithine)–INF-peptide have been shown to target hepatocytes *in vivo* [52]. An interesting system was described using a recombinant fusion peptide containing a Gal4 DNA-binding domain associated with a fragment of the invasin protein of *Yersinia pseudotuberculosis* [53]. The Gal4 part of the system confers both NLS and DNA-binding activities and invasin recognises integrin receptors at the surface of the cell [53,54••].

Most methods developed for gene delivery involve transport of genes of interest into cells via the endosomal pathway, which unfortunately leads to extensive degradation of the genes in lysosomal compartments and/or to their poor release into the cytoplasm. An interesting alternative is the design of peptide-based gene delivery systems that bypass the endosomal pathway or that fuse with the plasma membrane at neutral pH. Our group has developed a single-chain peptide vector, MPG (GALFLGFLGAAGSTMGAWSQPKSKRKV), that associates a fusion peptide sequence derived from the hydrophobic fusion peptide of HIV-1 gp41 and the hydrophilic NLS of Simian virus 40 large T antigen. MPG can efficiently deliver short oligonucleotides and larger plasmids into a wide variety of cell lines independently of the endosomal pathway [35,36••]. This peptide interacts strongly with nucleic acids through its NLS domain, and forms peptide–peptide interactions through the gp41 hydrophobic domain, thus generating a peptide cage around the plasmid. After crossing the cell membrane, the presence of the NLS domain promotes fast delivery of the plasmid into the nucleus. Structural and mechanistic investigations have revealed that the flexibility between the two domains of MPG is crucial for gene delivery and can be improved by adding a linker sequence between the fusion and the NLS motifs [35,36••,40,55].

An important problem to be addressed for gene delivery *in vivo* is the risk of interacting with serum proteins and cell surfaces in a non-specific manner because of the presence of positive charge at the surface of the peptide–DNA formulation. In order to limit these problems, various strategies have been proposed, for example, using polymer inducing steric hindrances in the formulation, such as polyethylene glycol (PEG), dextran, or poly-*N*-(2-hydroxypropyl) methacrylamide [8••,56]; the steric component helps by covering the surface of the peptide formulation and limiting non-specific ionic and hydrophobic interactions. A multivalent reactive polymer containing a poly-*N*-(2-hydroxypropyl) methacrylamide–tetrapeptide (GFLG) attached to a poly-L-lysine was shown to inhibit protein binding to the DNA–poly-L-lysine formulation and to improve gene delivery *in vitro* significantly [57].

Peptide combined with other delivery systems

In order to overcome the limitations of both peptide- and lipid-based delivery systems and to enhance gene transfer efficiency, peptides have been successfully incorporated into lipid-based or polymer-based delivery systems. Addition of lipid/polymer to peptide delivery systems improves the stability of the peptide–DNA complex and favor both DNA condensation and fusion with the membrane.

Peptides can be structurally engineered to impart very specific functions that lipid and polymer systems cannot provide efficiently, such as cell targeting, endosome lysis and nuclear localization. Peptides have been used to improve encapsulation efficiency of plasmids precondensed with short positively charged lipids [8••]. Synthetic peptides (e.g. JTS1 and INF) have been used to enhance the transfection efficiency of polymer-based systems [13,26,27]. Peptides containing DNA-binding and membrane-disrupting activities have been added into liposomes or lipid–plasmid complexes [26,27]. Peptides conferring cell specificity have also been used to target lipid–plasmid formulations. Recently, the DNA-condensing peptide (RGD-oligo-L-lysine) has been associated with cationic liposomes for gene delivery in tracheal epithelial cells [22].

Conclusions

An ideal non-viral system for *in vivo* gene delivery should be able to form reversible small and highly stable particles with DNA, to specifically target cells, to enhance cellular uptake and endosomal release, and to facilitate nuclear localisation of plasmids. In addition to these basic requirements, several other important parameters, such as the level and the persistence of expression of therapeutic proteins and the toxicity and the stability of the formulation *in vivo*, should be seriously taken into account for the design of a non-viral gene-delivery system. None of the synthetic DNA delivery systems described so far can satisfy all of these constraints. Peptide-based gene delivery technology is still in its early days in the field of gene therapy, however, and important progress made in the past five years in the understanding of the structural and mechanistic properties of peptides is providing great potential for the rational design of more efficient and yet-to-come synthetic peptides.

Two strategies using peptides are promising to reach the efficiency of viral gene delivery systems without their limitations in the future. Firstly, the development of multifunctional peptides or chimeric proteins that consist of several active domains; improvements should focus on the rapid release of DNA into the cytoplasm, cell targeting, selectivity and stability of the formulation *in vivo*. Secondly, the development of complex peptide–liposome or lipopeptide formulations; efforts should be made in optimising the structure and functionality of peptides.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- * of special interest
- ** of outstanding interest

1. Anderson WF: **Human gene therapy.** *Nature* 1998, **392**:25-30.

2. Ledley FD: **Nonviral gene therapy: the promise of genes as pharmaceutical products.** *Hum Gene Ther* 1995, **6**:1129-1144.

3. Luo D, Saltzman M: **Synthetic DNA delivery systems.**
** *Nat Biotechnol* 2000, **18**:33-37.
A recent review on the non-viral gene delivery systems giving advantages and limitations.

4. Mahato RI, Smith LC, Rolland A: **Pharmaceutical perspectives of nonviral gene therapy.** *Adv Genet* 1999, **41**:95-156.

5. Lee RJ, Huang L: **Lipidic vector systems for gene transfer.** *Crit Rev Ther Drug Carrier Syst* 1997, **14**:173-206.

6. Scherman D, Bessodes M, Cameraon B, Herscovici J, Hofland H, Pitard B, Soubrier F, Wils P, Crouzet J: **Application of lipids and plasmid design for gene delivery to mammalian cells.** *Curr Opin Biotechnol* 1998, **9**:480-485.

7. Boussif O, Lezoualc'h F, Zanta MA, Mergny M, Scherman D, Demeinex B, Behr JP: **A versatile vector for gene and oligonucleotide transfer into cells in culture and *in vivo*: polyethylenimine.** *Proc Natl Acad Sci USA* 1995, **92**:7297-7301.

8. Mahato RI, Monera OD, Smith LC, Rolland A: **Peptide-based gene**
** **delivery.** *Curr Opin Mol Ther* 1999, **1**:226-243.
An excellent review on peptide-based gene delivery, especially on condensing and endosomolytic peptides, intracellular trafficking and nuclear entry of plamids.

9. Bloomfield VA: **DNA condensation by multivalent cations.** *Biopolymers* 1998, **44**:269-282.

10. Wu GY, Wu CH: **Receptor-mediated *in vitro* gene transformation by a soluble DNA carrier system.** *J Biol Chem* 1987, **262**:4429-4432.

11. Tang MX, Szoka FC Jr: **The influence of polymer structure on the interactions of cationic polymers with DNA and morphology of the resulting complexes.** *Gene Ther* 1997, **4**:823-832.

12. Abdallah B, Hassan A, Benoist C, Goula D, Behr JP, Demeneix BA: **A powerful nonviral vector for *in vitro* gene transfer into the adult mammalian brain: polyethyleneimine.** *Hum Gene Ther* 1996, **7**:1947-1954.

13. Gottschalk S, Sparrow JT, Hauer J, Mims MP, Leland FE, Woo SL, Smith LC: **A novel DNA–peptide complex for efficient gene transfer and expression in mammalian cells.** *Gene Ther* 1996, **3**:448-457.

14. Kabanov AV, Kabanov VA: **DNA complexes with polycations for the delivery of genetic material into cells.** *Bioconjug Chem* 1995, **6**:7-20.

15. Plank C, Tang MX, Wolfe AR, Szoka FC Jr: **Branched cationic**
** **peptides for gene delivery: role of type and number of cationic residues in formation and *in vitro* activity of DNA polyplexes.** *Hum Gene Ther* 1999, **10**:319-332.
This paper details the design of synthetic branched cationic peptides for condensing DNA and gene delivery.

16. Smith LC, Duguid JG, Wadhwa MS, Logan MJ, Tung CH, Edwards V, Sparrow ST: **Synthetic peptide-based DNA complexes for non viral gene delivery.** *Adv Drug Deliv Rev* 1998, **30**:115-131.

17. Blessing T, Remy JS, Behr JP: **Monomolecular collapse of plasmid DNA into stable virus-like particles.** *Proc Natl Acad Sci USA* 1998, **95**:1427-1431.

18. Wadhwa MS, Collard WT, Adami RC, McKenzie DL, Rice KG: **Peptide mediated gene delivery influence of peptide structure on gene expression.** *Bioconj Chem* 1997, **8**:81-88.

19. McKenzie DL, Kwok KY, Rice KG: **A potent new class of reductively activated peptide gene delivery agents.** *J Biol Chem* 2000, **275**:9970-9977.

20. Harbottle RP, Cooper RG, Hart SL, Ladhoff A, McKay T, Knight AM, Wagner E, Miller AD, Coutelle C: **An RGD-oligolysine peptide: a prototype construct for integrin-mediatad gene delivery.** *Hum Gene Ther* 1998, **9**:1037-1047.

21. Pasqualini R, Koivunen E, Ruoslahti E: **αv integrins as receptors for tumor targeting by circulating ligands.** *Nat Biotechnol* 1997, **15**:542-546.

22. Colin M, Maurice M, Trugnan G, Kornprobst M, Harbottle RP, Knight A, Cooper RG, Miller AD, Capeau J, Coutelle C *et al.*: **Cell delivery, intracellular trafficking and expression of an integrin-mediated gene transfer vector in tracheal epithelial cells.** *Gene Ther* 2000, **7**:139-152.

23. Plank C, Zatloukal K, Cotten M, Mechtler K, Wagner E: **Gene transfer into hepatocytes using asialoglycoprotein receptor mediated endocytosis of DNA complexed with an artificial tetra-antennary galactose ligand.** *Bioconjug Chem* 1992, **3**:533-539.

24. Ferkol T, Perales JC, Mularo F, Hansen RW: **Receptor-mediated gene transfer into macrophages.** *Proc Natl Acad Sci USA* 1996, **93**:101-105.

25. Schuster MJ, Wu GY, Walton CM, Wu CH: **Multicomponent DNA carrier with a vesicular stomatitis virus G-peptide greatly enhances liver-targeted gene expression in mice.** *Bioconjug Chem* 1999, **10**:1075-1083.

26. Wagner E: **Effects of membrane-active agents in gene delivery.** *J Controlled Release* 1998, **53**:155-158.

27. Plank C, Zauner W, Wagner E: **Application of membrane-active peptides for drug and gene delivery across cellular membrane.** *Adv Drug Deliv Rev* 1998, **34**:21-35.

28. Lear JD, DeGrado WF: **Membrane binding and conformational properties of peptides representing the NH2 terminus of influenza HA-2.** *J Biol Chem* 1987, **262**:6500-6505.

29. Plank C, Oberhauser B, Mechtler K, Koch C, Wagner E: **The influence of endosome-disruptive peptides on gene transfer using synthetic virus-like gene transfer system.** *J Biol Chem* 1994, **269**:12918-12924.

30. Wagner E, Plank C, Zatloukal K, Cotten M, Birnstiel ML: **Influenza virus hemagglutinin HA-2 N-terminal fusogenic peptides augment gene transfer by transferrin-polylysine-DNA complexes: toward a synthetic virus-like gene transfer vehicle.** *Proc Natl Acad Sci USA* 1992, **89**:7934-7938.

31. Parente RA, Nadeasdi L, Subabrao NK, Szoka FC Jr: **Association of a pH-sensitive peptide with membrane vesicles: role of amino-acid sequence.** *Biochemistry* 1990, **29**:8713-8719.

32. Kichler A, Mechtler K, Behr JP, Wagner E: **Influence of membrane-active peptides on lipospermine/DNA complex mediated gene transfer.** *Bioconjug Chem* 1997, **8**:213-221.

33. Haensler J, Szoka FC Jr: **Polyamidoamine cascade polymers mediate efficient transfection of the cell in culture.** *Bioconjug Chem* 1993, **4**:372-379.

34. Midoux P, Kichler A, Boutin V, Maurizot JC, Monsigny M: **Membrane permeabilization and efficient gene transfer by a peptide containing several histidines.** *Bioconjug Chem* 1998, **9**:260-267.

35. Morris MC, Vidal P, Chaloin L, Heitz F, Divita G: **A new peptide vector for efficient delivery of oligonucleotide into nontransformed mammalian cells.** *Nucleic Acids Res* 1997, 25:2730-2736.

36. •• Morris MC, Chaloin L, Mery J, Heitz F, Divita G: **A novel potent strategy for gene delivery using a single peptide vector as a carrier.** *Nucleic Acids Res* 1999, 27:3510-3517.
This paper describes a new single chain peptide derived from both the nuclear localisation sequence of SV40 and the fusion peptide domain of HIV-1 gp41. This peptide forms stable complexes with DNA and delivers plasmids into cells independently of the endosomal pathway. In this work, it was used for the targeting of an essential cell-cycle gene encoding for the phosphatase cdc25C.

37. Görlich D, Mattaj IW: **Nucleocytoplasmic transport.** *Science* 1996, 271:1513-1518.

38. Mattaj IW, Englmeier L: **Nucleocytoplasmic transport: the soluble phase.** *Annu Rev Biochem* 1998, 123:265-306.

39. Goldfarb DS, Gariepy J, Schoolnik G, Kornberg RD: **Synthetic peptides as nuclear localization signals.** *Nature* 1986, 322:614-644.

40. Chaloin L, Vidal P, Heitz A, Nguyen Van Mau N, Mery J, Divita G, Heitz F: **Conformations of primary amphipathic carrier peptides in membrane mimicking environments.** *Biochemistry* 1997, 36:11179-11187.

41. Sebestyen MG, Ludtke JJ, Bassik MC, Zhang G, Budker V, Lukhtanov EA, Hagstrom JE, Wolff JA: **DNA vector chemistry: the covalent attachment of signal peptides to plasmid DNA.** *Nat Biotechnol* 1998, 16:80-85.

42. Zanta MA, Belguise-Valladier P, Behr JP: **Gene delivery: a single nuclear localization signal peptide is sufficient to carry DNA to the cell nucleus.** *Proc Natl Acad Sci USA* 1999, 96:91-96.

43. Ciolina C, Byk G, Blanche F, Thuillier V, Scherman D, Wils P: **Coupling of nuclear localization signales to plasmid DNA and specific interaction of the conjugates with importin α.** *Bioconjug Chem* 1999, 10:49-55.

44. Wilke M, Fortunati E, Van Den Broek M, Hoogeveen AT, Scholte BJ: **Efficacy of a peptide-based gene delivery system depends on mitotic activity.** *Gene Ther* 1996, 3:1133-1142.

45. Legendre JY, Szoka FC: **Cyclic amphipathic peptide–DNA complexes mediate high efficiency transfection of adherent mammalian cells.** *Proc Natl Acad Sci USA* 1993, 90:893-897.

46. Wyman TB, Nicol F, Zelphati O, Scaria PV, Plank C, Szoka FC Jr: **Design, synthesis and characterization of a cationic peptide that binds to nucleic acids and permeabilizes bilayers.** *Biochemistry* 1997, 27:3008-3017.

47. Niidome T, Ohmori N, Ichinose A, Wada A, Mihara H, Harayama T, Aoyagi H: **Binding of cationic α-helical peptides to plasmid DNA and their gene transfer abilities into cells.** *J Biol Chem* 1997, 272:15307-15312.

48. •• Niidome T, Takaji K, Urakawa M, Ohmori N, Wada A, Harayama T, Aoyagi H: **Chain length of cationic α-helical peptide sufficient for gene delivery into cells.** *Bioconjug Chem* 1999, 10:115-120.
This paper describes a detailed characterisation of cationic α-helical amphipathic peptides. The authors investigate the impact of the sequence, length, and structure of the peptides on DNA binding and gene delivery.

49. Midoux P, Monsigny M: **Efficient gene transfer by histidylated polylysine/pDNA complexes.** *Bioconjug Chem* 1999, 10:400-411.

50. •• Avrameas A, Temynck T, Gasmi L, Buttin G: **Efficient gene delivery by a peptide derived from monoclonal anti-DNA antibody.** *Bioconjug Chem* 1999, 10:87-93.
The authors demonstrate of the significant enhancement of cell targeting by a single peptide containing an anti-DNA antibody.

51. •• Schuster M, Wu GY, Walton CM, Wu CH: **Multicomponent DNA carrier with a vesicular stomatitis virus G-peptide greatly enhances liver-targeted gene expression in mice.** *Bioconjug Chem* 1999, 10:1075-1083.
This paper describes an interesting approach for *in vivo* gene delivery based on a multicomponent DNA carrier containing an asialoorosomucoid-poly-L-lysine associated with a lytic peptide derived from the vesicular stomatitis virus glycoprotein.

52. Toncheva V, Wolfert MA, Dash PR, Oupicky D, Ulbrich K, Seymour LW, Schacht EH: **Novel vectors for gene delivery formed by self assembly of DNA with poly(L-lysine) grafted with hydrophilic polymers.** *Biochim Biophys Acta* 1998, 1380:354-368.

53. Chan CK, Hubner S, Hu W, Jans DA: **Mutual exclusivity of DNA binding and nuclear localization signal recognition by the yeast transcription factor GAL4: implications for non-viral DNA delivery.** *Gene Ther* 1998, 5:1204-1212.

54. •• Nishikawa M, Yamauchi M, Morimoto K, Ishida E, Takakura Y, Hashida M: **Hepatocyte-targeted *in vivo* gene expression by intravenous injection of plasmid DNA complexed with synthetic multi-functional gene delivery system.** *Gene Ther* 2000, 7:548-555.
This paper describes the use of a multicomponent cationic polymer, galactose-poly(L-ornithine)-fusigenic peptide, for *in vivo* gene delivery into hepatocytes.

55. Vidal P, Chaloin L, Heitz A, Van Mau N, Mery J, Divita G, Heitz F: **Interaction of primary amphipatic vector peptides with membranes: conformational consequences and influence on cellular localisation.** *J Membr Biol* 1998, 162:259-264.

56. Hara T, Lui DX, Huang L: **Emulsion formulations as a vector for gene delivery *in vitro* and *in vivo*.** *Adv Drug Deliv Rev* 1997, 24:265-271.

57. Dash PR, Read ML, Fisher KD, Howard KA, Wolfert M, Oupicky D, Subr V, Strohalm J, Ulbrich K, Seymour LW: **Decreased binding of proteins and cells of polymeric gene delivery vector surface modified with a multivalent hydrophilic polymer and targeting through attachment of tranferrin.** *J Biol Chem* 2000, 275:3793-3802.